MetaXpress® 6 FAQ

Optimizing Exposure Times

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Frequently Asked Questions Overview

The purpose of this FAQ guide is to help the user optimize exposure times during acquisition.

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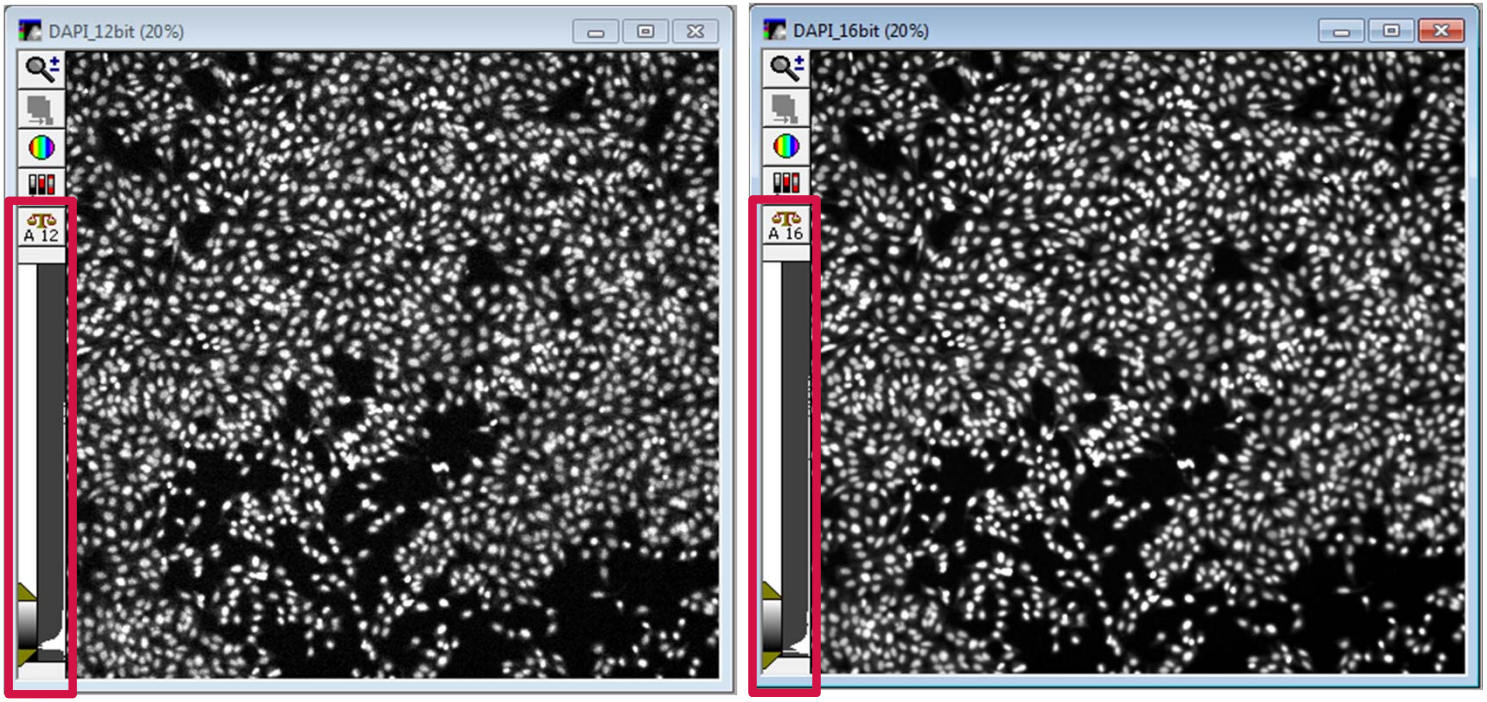
Suggested Exposure Time

1. Molecular Devices recommends setting exposure times to ~50-75% of the bit range of your camera
   - Some ImageXpress Micro systems have a 12-bit CCD camera (4095 gray levels) and others have a 16-bit sCMOS camera (65,535 gray levels)
   - In the main toolbar, right-click in the empty space and select **Camera state**. Click on **Info** icon to determine which camera your system has



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What is Camera / Image Bit Depth?

- Bit depth defines the number of grey values available to each pixel within an image
- Bit depth determines the maximum intensity that can be displayed at one time
- Also called "color depth" and "pixel depth"

Bit depth	Number of colors
1-bit	Black and white
4-bits	16 (Standard VGA)
8-bits	256 (Super VGA, indexed color)
8-bit grayscale	256 shades of gray
12-bit grayscale	4095 shades of gray
16-bits	65,536 (High color) shades of gray
24-bits	16.7 million colors (True color)
32-bits	16,777,216 + 256 levels of transparency

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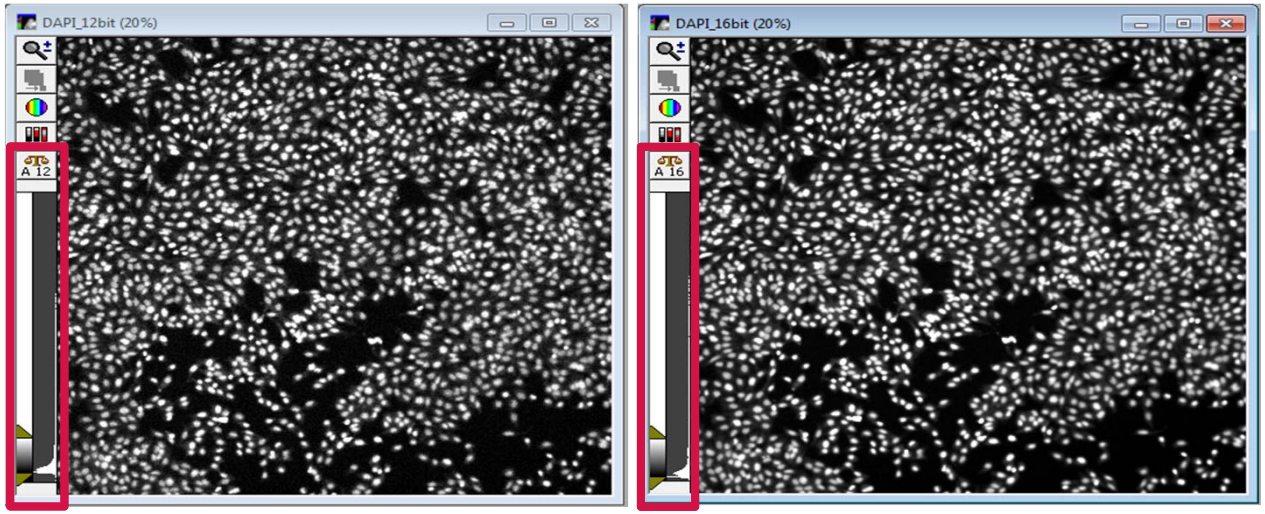
How Do I Optimize Exposure Times?

2. To determine optimal exposure times during set up of your acquisition settings
   - Go to a control well with brightest signal and click on the **Focus** button
   - On the appropriate **W** tab, set **Target Max Intensity** to **3000** for a 12-bit camera and **33000** for a 16-bit camera and click the **Auto Expose** button, OR
   - Manually adjust exposure time until the Scale icon displays 12- or 16- bit and the histogram shows pixel intensities reaching 50-75% of the scale on the **Image** window
   - Check the exposure time on a well with dim signal – a well where you expect to detect objects that have low intensity. Adjust exposure time, if necessary, so that objects on the dim well can be detected and objects in the bright well are not saturated (see section on Saturated Images).



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Determining Bit Depth Level of an Image

All images taken on the ImageXpress systems are saved as 16-bit. However, the displayed gray level is dependent on the camera and exposure time. The images on the right are the same sample imaged at different exposure times to yield the displayed bit-depths. The bit-depth level of the image can be determined by clicking the **Image Scale** Icon and selecting **Best Fit Range**.

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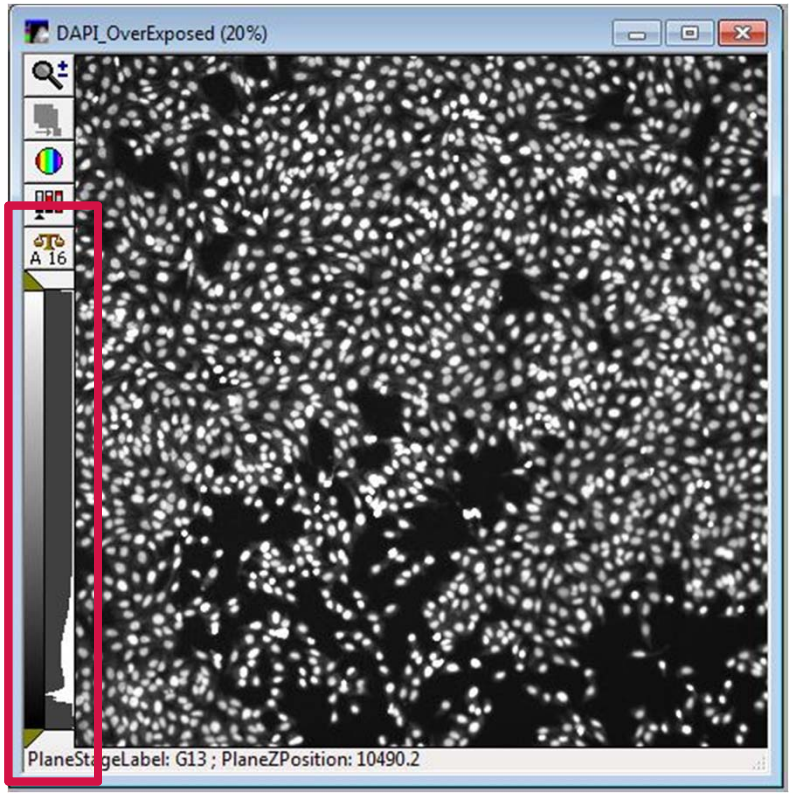
Saturated Images

In some cases, a saturated image is easy to spot based on what the objects look like in the image (certain objects look very bright, out of focus, or blob-like). In other cases, as shown below, it is not as apparent. To determine if an image is saturated, examine the histogram. Saturated images will show the histogram at 100%. Reduce the exposure time as necessary.



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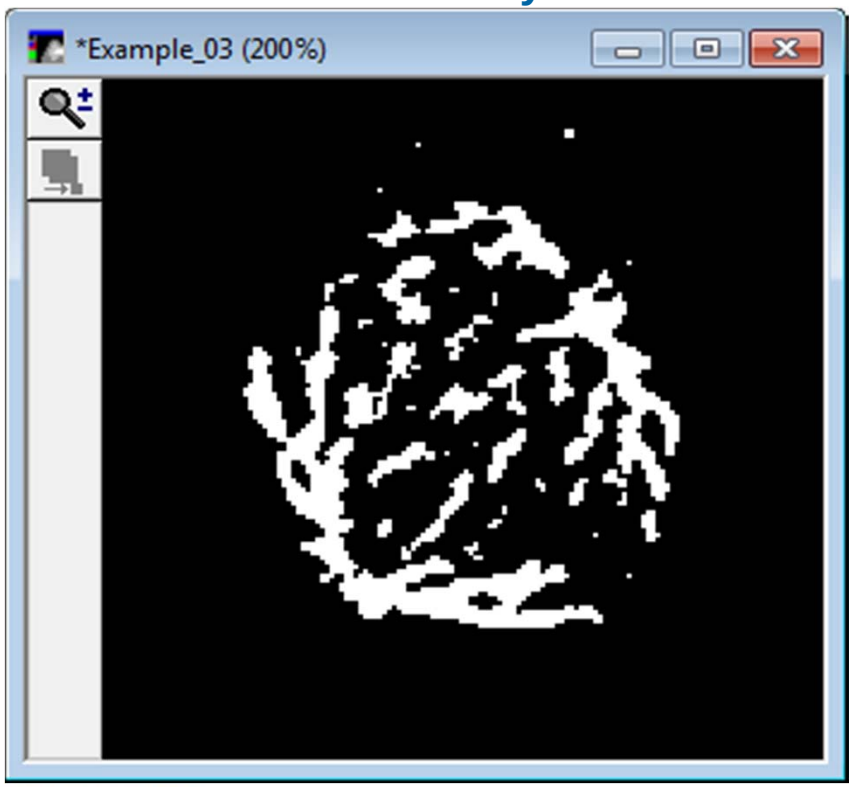
Other Bit Depths

- 1-bit binary images are typically produced when images are thresholded or segmented and that threshold/segmentation is converted to an image

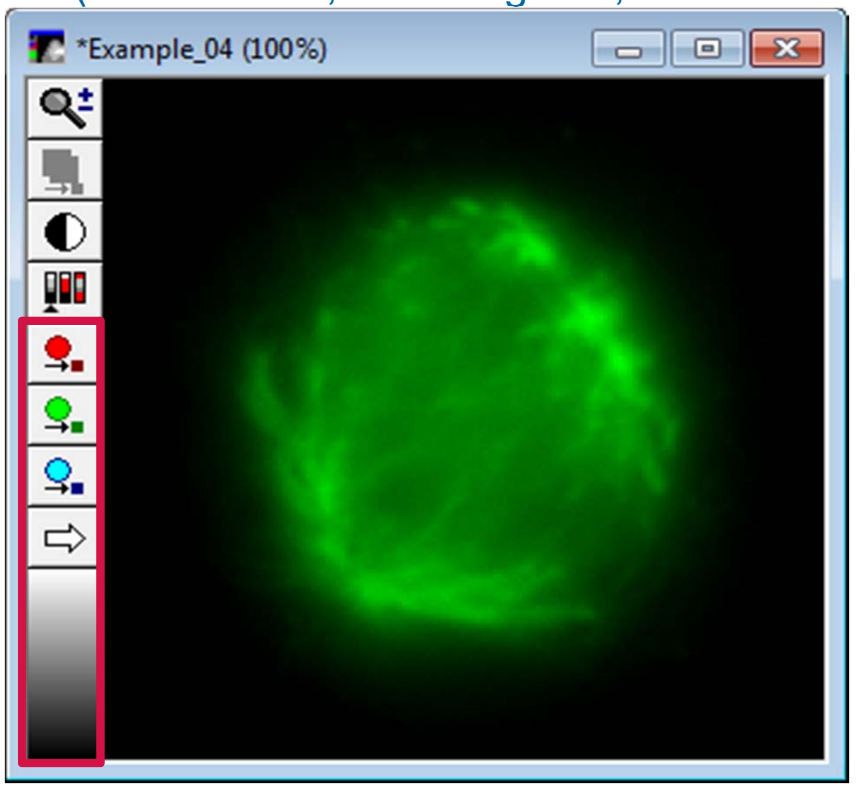
- 24-bit images are the result of a color overlay

1 Bit "Binary"



24 Bit

(8 bits for red, 8 bit for green, 8 bit for



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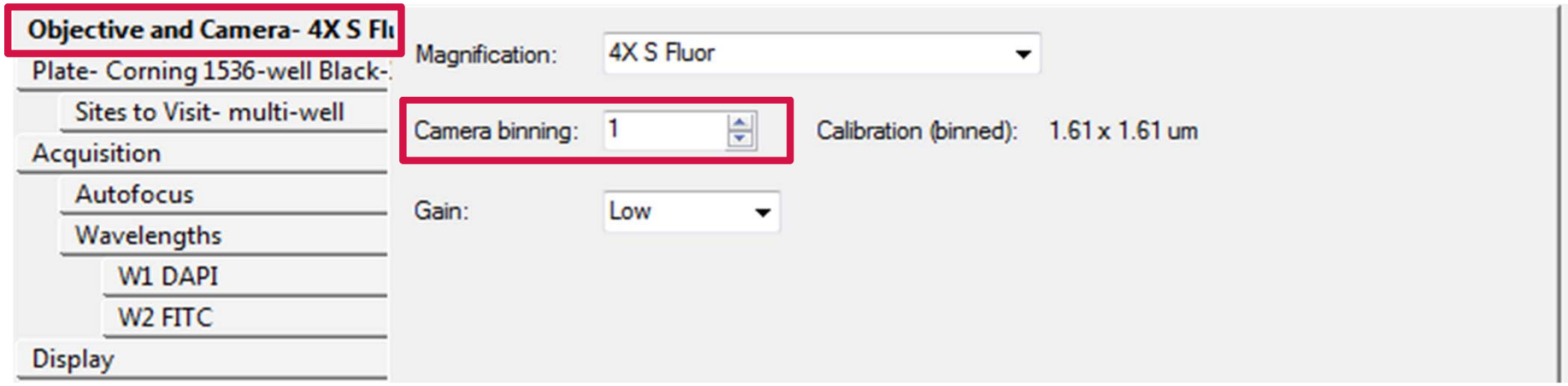
A Note About Binning

3. On the **Objective and Camera** tab, you have the option to bin. You may want to bin depending on your analysis and image resolution needs (see next section on Binning)
   - Increasing binning (≥2) will result in a lower resolution image but higher intensity for each pixel
   - Therefore, it may be necessary to reduce exposure time for each wavelength acquired



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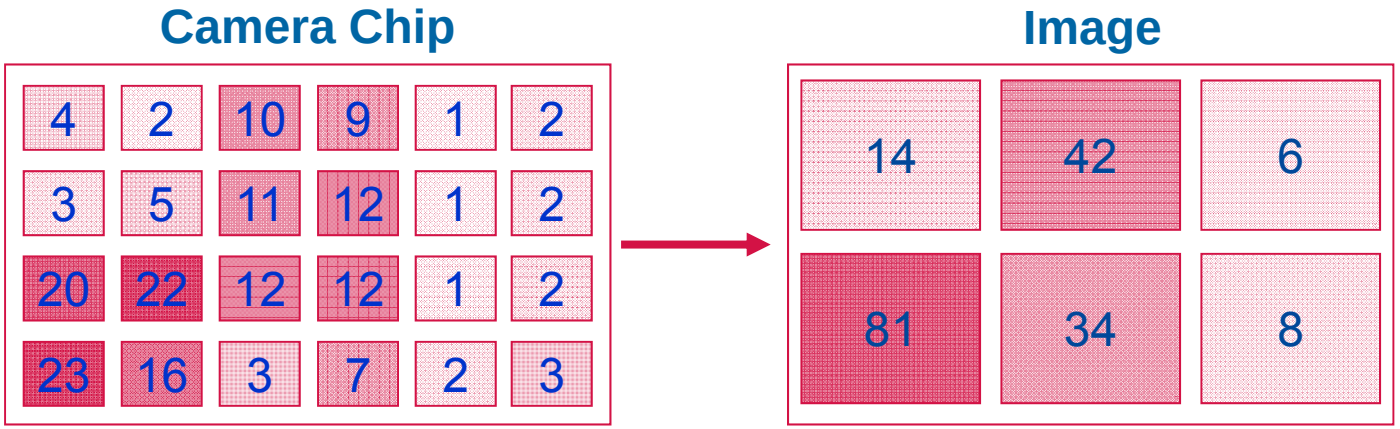
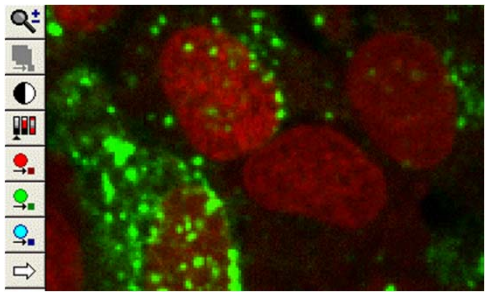
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

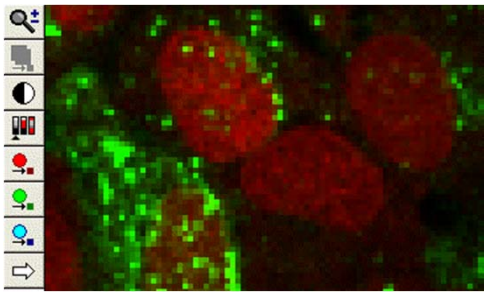
Example of 2x2 Binning



Each pixel records an intensity



4 Pixels are summed to make one larger pixel



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Why Bin?

Brighter pixels

- The resultant pixel is brighter than any of the 4 component pixels

Save Space

- 2x2 binning reduces file size 4-fold

Increase Speed

- Faster image transfer from MetaXpress to database
- Faster image analysis

When to Bin

- You do not need to see intricate sub-cellular detail
- Cell counting
- Scoring cells positive or negative for fluorescent markers
- Measuring overall cell intensity

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Exceptions to Optimal Exposure Times

4. There are some exceptions to the recommendations for exposure times that depend on your sample and analysis goals:
   - Intensities can be set to < 50% of scale when the goal is to only count objects and not compare intensity values
   - Some samples may be photosensitive or photobleach quickly and therefore require lower exposure times (especially important for Time lapse and Z Series acquisition)
   - To reduce exposure times in order to increase the speed of your assay

5. Suggested Minimum Exposure Times:
   - Counting Objects
     - 12-bit camera: within 50% of 8-bit intensity levels (> 125)
     - 16-bit camera: within 50% of 12-bit intensity levels (> 2000)
   - Comparing Intensity
     - 12-bit camera: within 50% of 10-bit intensity levels (> 500)
     - 16-bit camera: within 25% of 14-bit intensity levels (> 4000)

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Example: Reducing Exposure Time

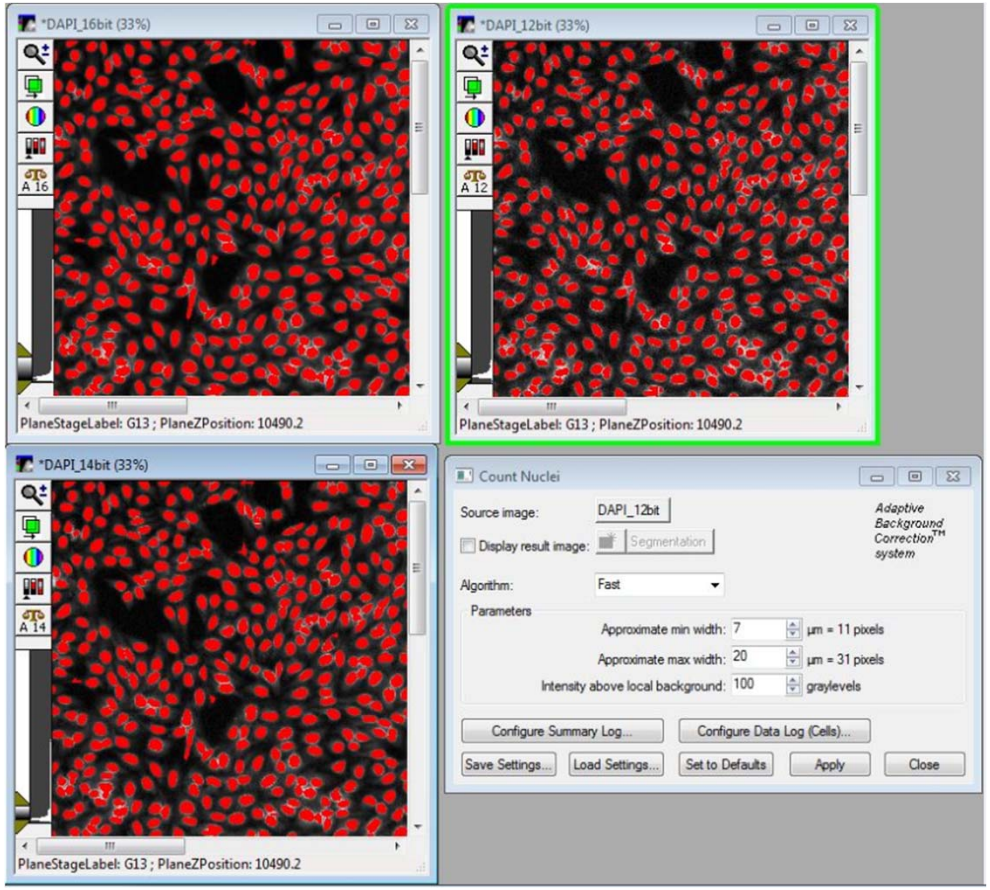
In this example, the same sample was imaged using the following settings:

- 7 ms for 12-bit pixel intensity range
- 20 ms for 14-bit pixel intensity range
- 130 ms for 16-bit pixel intensity range

The **Count Nuclei** application module was used to determine nuclear counts in these images

- For the 12 and 14 bit images:
  - Min width = 7
  - Max width = 20
  - Threshold = 100
- For the 16 bit images:
  - Min width = 7
  - Max width = 20
  - Threshold = 500

Nuclear count = 1904 for all images



As this example shows, you can reduce the exposure time and still get the same analysis results with optimized analysis settings

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Image Scaling: Adjusting the Display

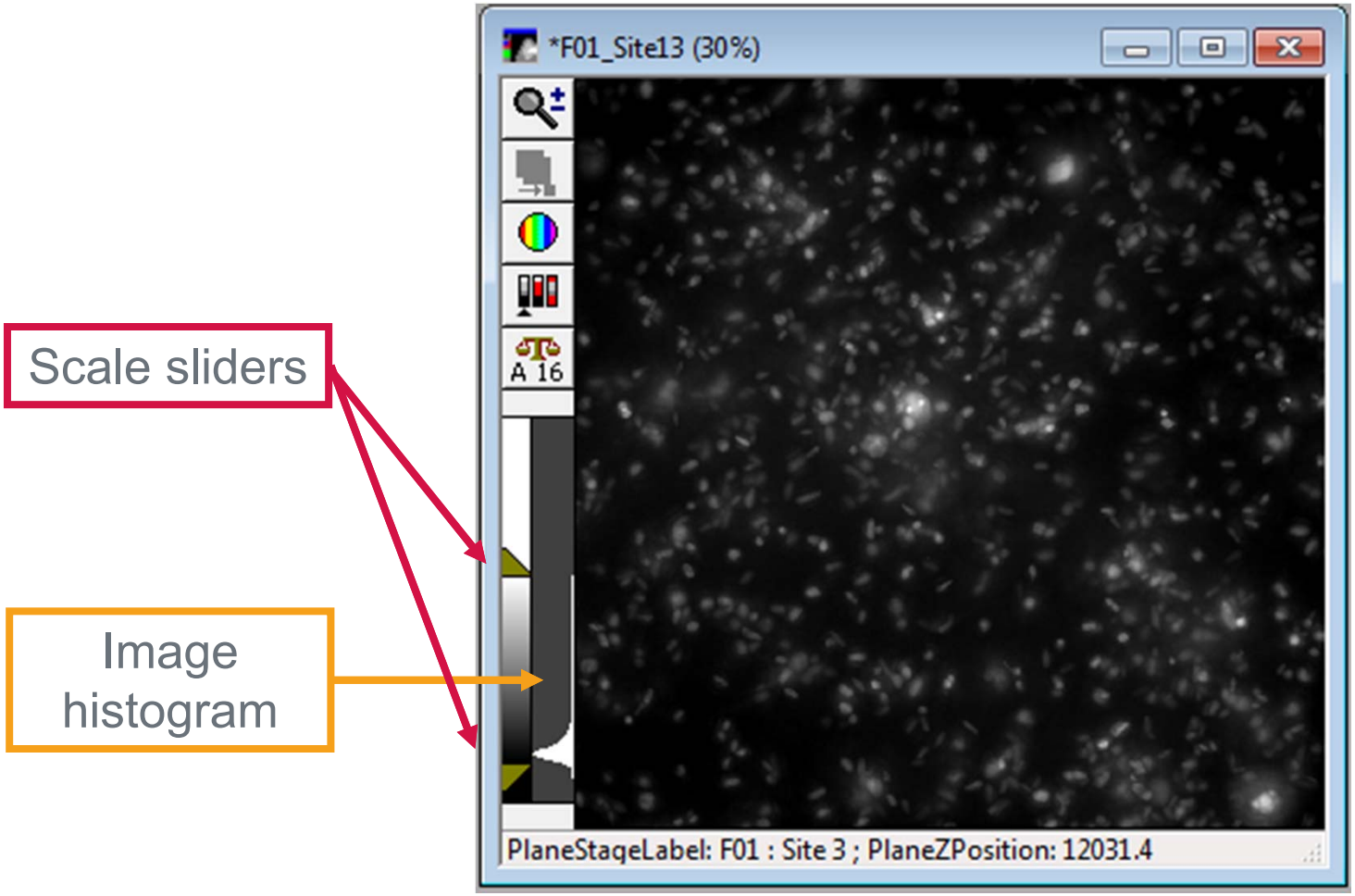

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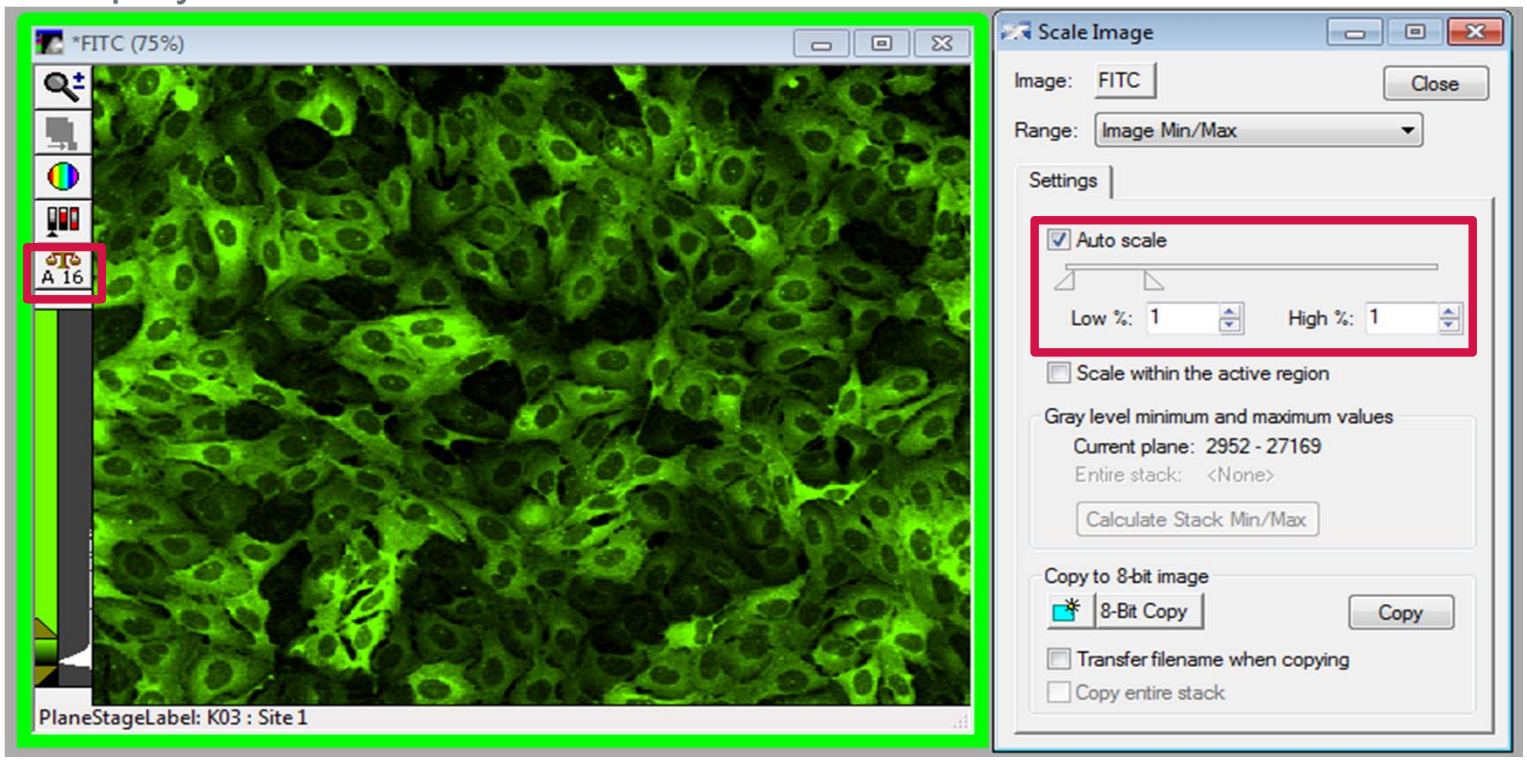
Image Scaling: Auto Scale Turned On

Click on the Scale Icon and select **Scale Image**

- When Auto Scale is on as seen in the image below, pixels in the upper 1% of the intensity range will be assigned the maximum possible intensity (usually 65,535) and pixels in the lower 1% of the intensity range will be assigned the minimum possible value (0, or black) therefore making the image brighter on the display.



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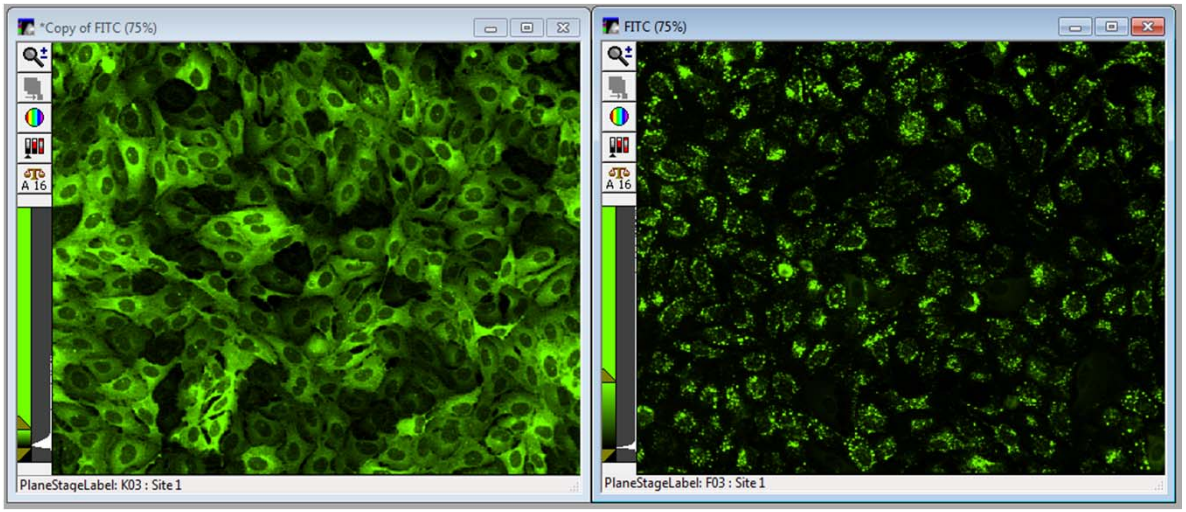
What Does Auto Scale Do?

Auto scale automatically adjusts the scaling based on the minimum and maximum intensities in each image.

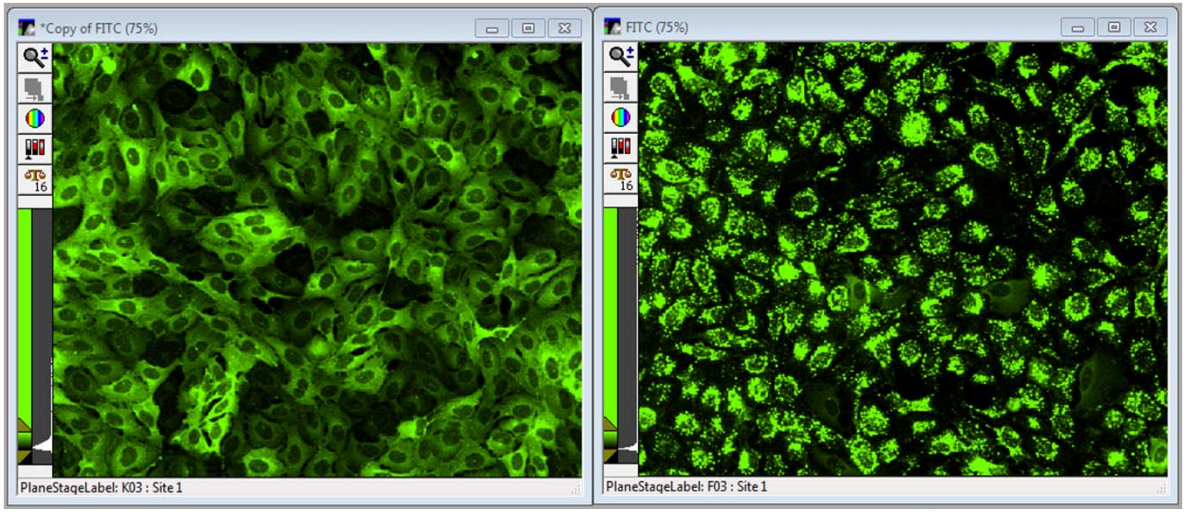
When comparing two images:

- Auto Scale ON: the images will appear with the same brightness
- Auto Scale OFF: the images will appear as their true intensity levels indicate

Auto Scale On



Auto Scale Off



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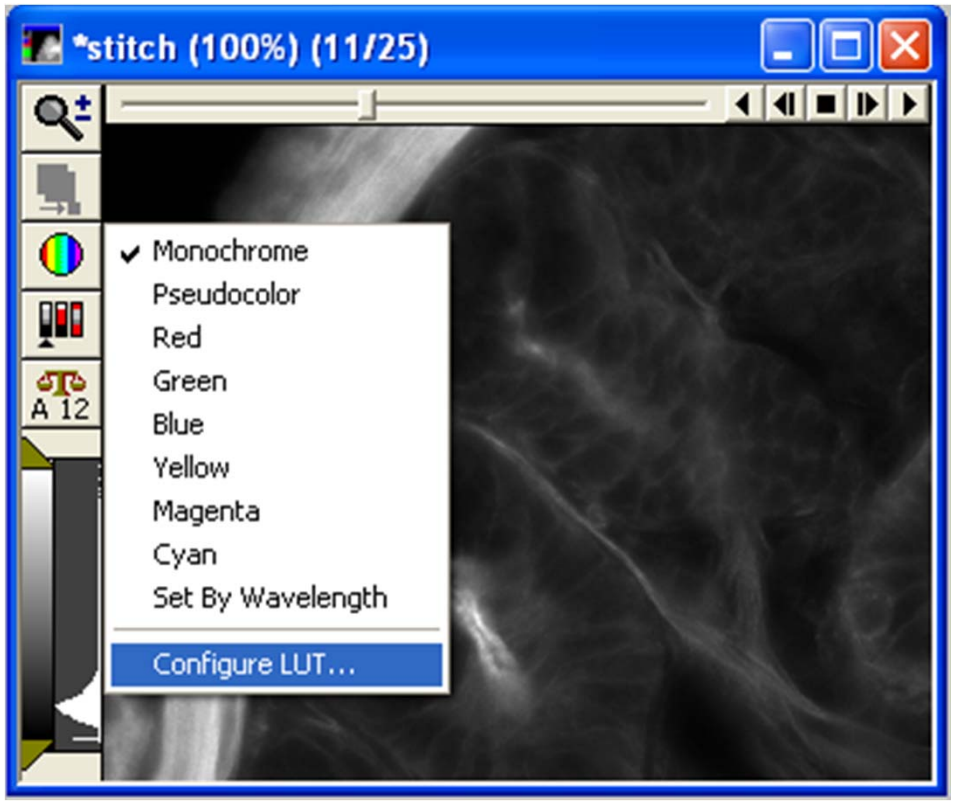
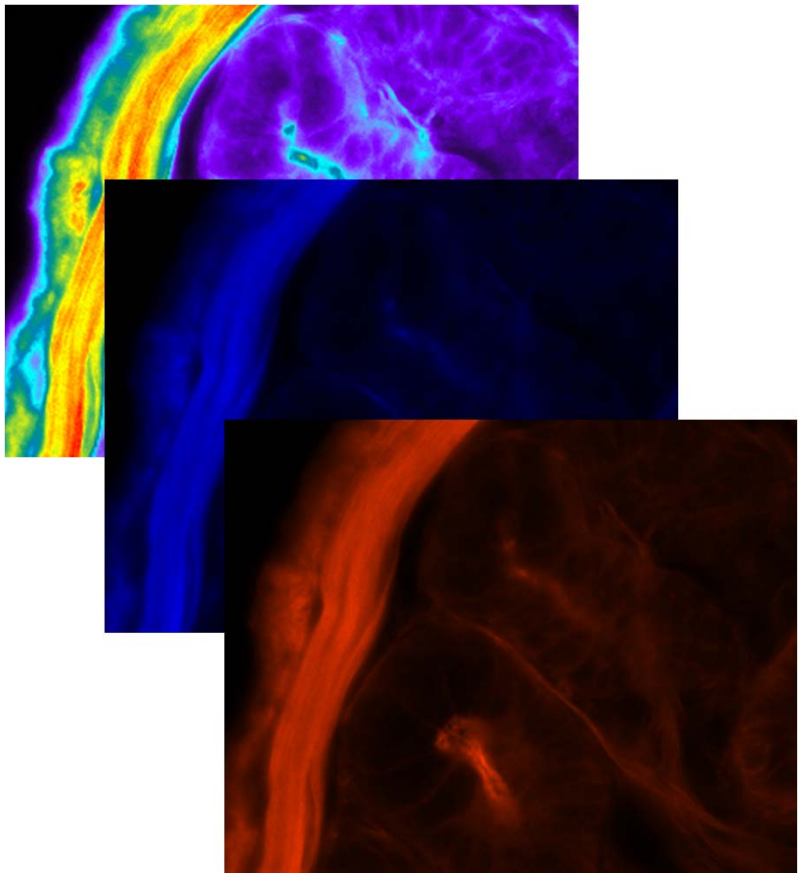
Changing the Displayed Color Through Look Up Tables (LUT)

Click on the icon to change the color of the image. This maps intensity values in image to a color but does not change the raw values.

- Pseudocolor: color appears according to intensity
- Monochrome: black and white





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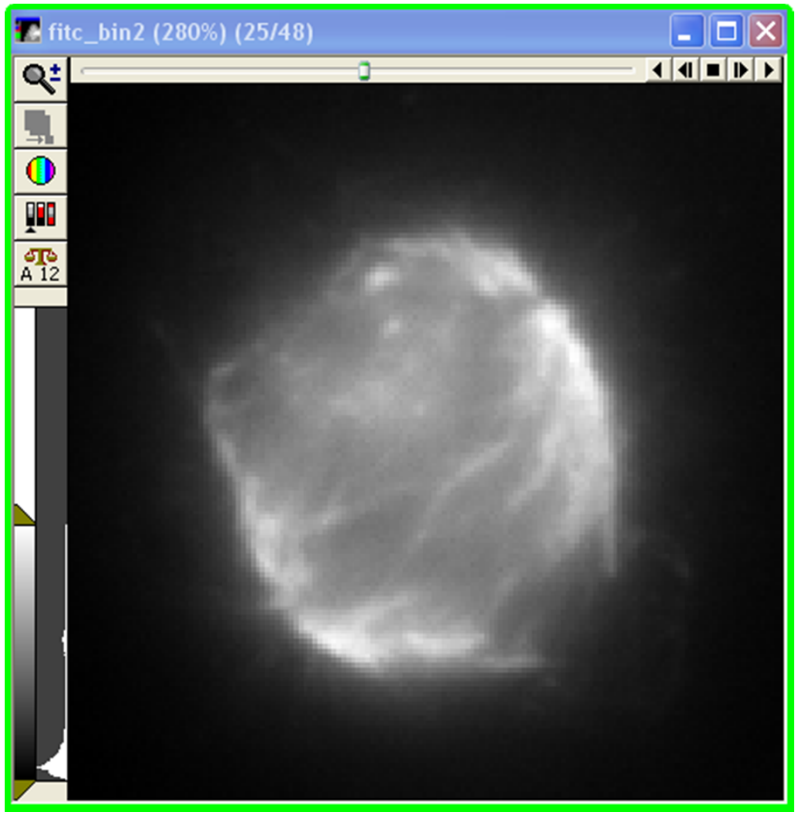
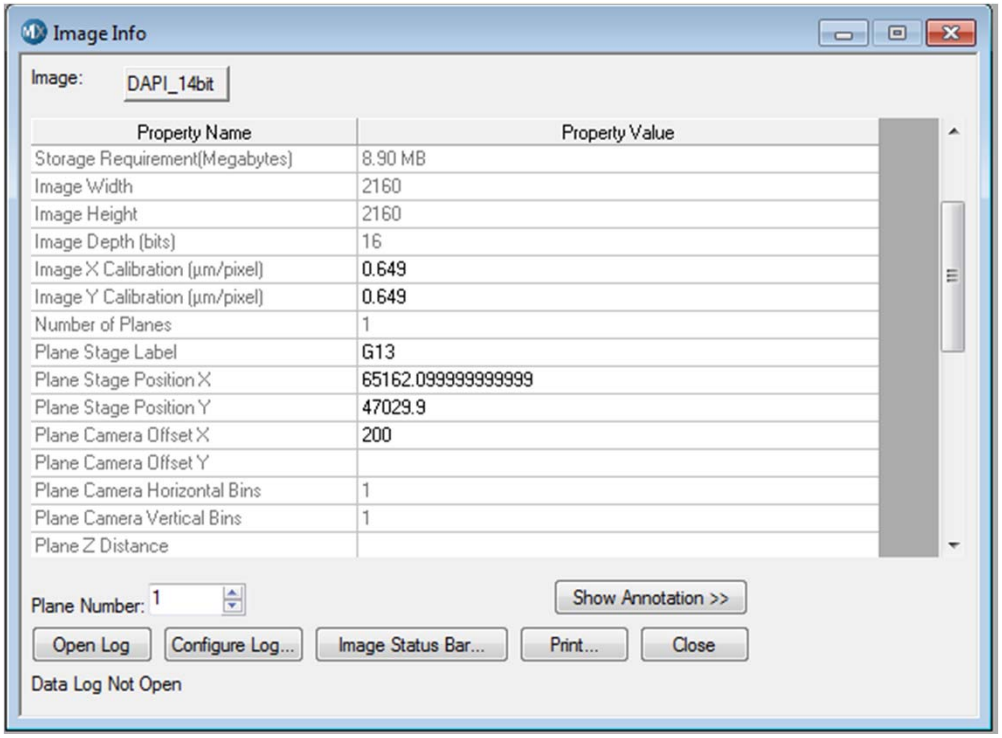
Viewing Image Information (Meta Data)

To open the **Image Info** dialog:

- In the main toolbar, select **Edit > Image > Image Info**
- Click on
- Press the **ALT + I** buttons on the key board





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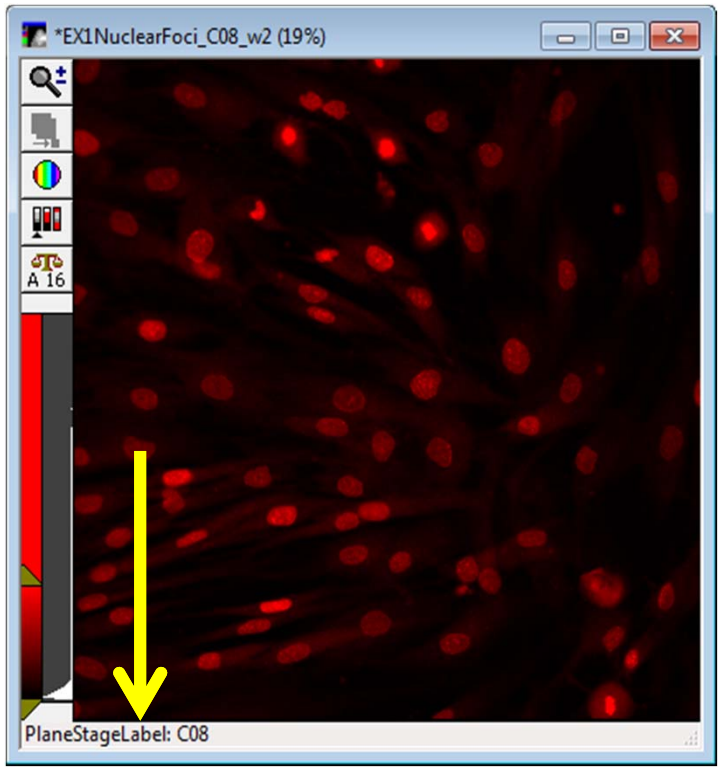
Add Image Information to Bottom of Image Window

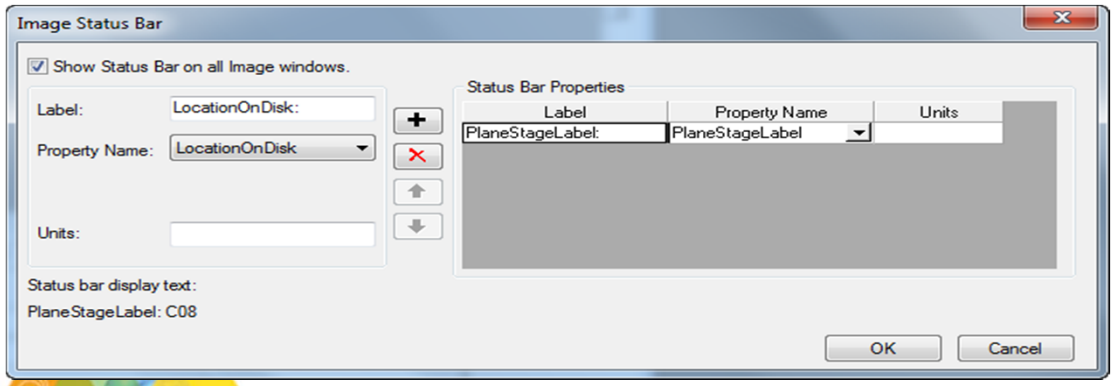


- In the main toolbar, select **Edit > Image > Image Status Bar**

  OR

- On the Image Info screen, click on **Image Status Bar** button

- Choose the information you would like to see under the **Property Name** drop-down menu and click the **+** button



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Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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